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COMMENTARY

DOES DEOXYGLUCOSE UPTAKE IN THE BRAIN REFLECT ENERGY METABOLISM?

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The question posed in the title can be answered unequivocally: The deoxyglucose method does not purport to measure the energy metabolic rate of brain tissue under all circumstances. Rather, Sokoloff intended the method to reveal functional activity of nerve cells under physiological circumstances with the resolution offered by autoradiography, and he consistently referred to the method as "functional mapping". There is compelling evidence that regeneration of ATP for the Na^+-K^+-ATPase of nerve cells proceeds by oxidative phosphorylation fuelled by glucose and determined by the activities of phosphofructokinase and hexokinase [1].

It is 11 years since the appearance of the first major discussion of the "2DG" method [2]. The discussion actually preceded Sokoloff's personal, complete presentation of the method [3] by an entire year, but more or less brief messages had been given to conferences and seminars since 1974 [4–8]. Thus, the method was widely known and debated years before the complete theoretical foundation was published. As the fame of the method grew, so did its peculiar reputation for obscurity that stemmed from ignorance of the complete derivation of the operational equations of the model.

The basis of the method is a simple biochemical principle: If the product of a biochemical reaction remains permanently trapped as a record of the activity of that reaction, if the reaction rate is independent of the precursor concentration and constant during the period of observation, then the rate of the reaction is the ratio between the accumulated product and the time–concentration integral of the precursor [9],

$$V_d k_3 = \frac{M_m(T)}{\int_0^T C_e(t)\,dt} \qquad (1)$$

where $M_m(T)$ is the accumulated product at the end of the period of observation, V_d the physical distribution volume of the precursor in brain, often equal to the water volume of brain, k_3 the rate of the reaction from precursor to product, and $C_e(t)$ the concentration of the precursor as a function of time.

Sokoloff's unique and outstanding contribution consists in (1) identifying a substrate for the brain hexokinase reaction that fulfilled the requirements for use of the equation; (2) identifying a principle useful to other cerebrovascular problems, including transfer across the blood–brain barrier, blood flow, protein synthesis, receptor–ligand interactions, and a host of yet unidentified reactions that may be described by simple compartment models; and (3) bringing together the principle embodied in the equation with the practice of autoradiography of the brain, an accomplishment which immediately brought the method to the forefront of neurobiology where crude neurochemical methods had ceased to be functionally meaningful. There is a continuous line of reasoning from the development of autoradiographic methods of blood flow measurement via the neurochemistry of brain metabolism to the functional mapping of the brain which deoxyglucose made possible.

Many attempts have been made to extend the usefulness of the method beyond that outlined in the original paper and much knowledge and debate have evolved from these attempts but, rather than detract from the original description, these attempts have grown into an enormously fruitful and provocative branch of neurobiology from which laboratories around the world have benefited.

Properties of deoxyglucose

Sokoloff identified 2-deoxyglucose as a substrate for the hexokinase reaction, the product of which (2-deoxyglucose-6-phosphate) remains trapped in brain because glucose phosphate isomerase rejects 2-deoxyglucose-6-phosphate and because the brain is peculiarly slow to dephosphorylate the product. This peculiarity is due to the paucity (and inaccesibility) of glucose-6-phosphatase in significant amounts [10–12]. Following Sokoloff's original choice of 2-deoxyglucose, the labels of certain other tracer hexoses were subsequently shown also to enjoy long retention times in brain, including fluorodeoxyglucose and perhaps glucose itself labeled in the carbon-6 position [13, 14].

Since the integrated precursor pool radioactivity and the accumulated metabolites are not separately measurable by autoradiography or whole-brain analyses, Sokoloff *et al.* [3] substituted the precursor pool integral in Equation 1 by the plasma integral to

exclude the immeasurable quantity $C_e(t)$,

$$V_f k_3 = \frac{M(T) - \dfrac{M_e(T) - k_4 \int_0^T M_m(t)\,dt}{1 + \dfrac{k_3}{k_2}}}{\int_0^T C_p(t)\,dt} \quad (2)$$

where $M(T)$ is the total radioactivity recorded in brain, $M_e(T)$ the radioactivity recorded in the precursor pool, and $M_m(t)$ the radioactivity accumulated in the product as a function of time. The term $C_p(t)$ represents the radioactivity in arterial plasma as a function of time. The coefficients k_2, k_3, and k_4 represent the rates of efflux from brain, phosphorylation, and dephosphorylation, respectively, and V_f is the actual (rather than physical) distribution volume of deoxyglucose in brain, equal to the ratio $K_1/(k_2 + k_3)$, where K_1 is the clearance of deoxyglucose from blood to brain. The product $V_f k_3$ represents the rate of "unidirectional" formation of deoxyglucose-6-phosphate, measured as a clearance from the circulation.

Sokoloff *et al.* [3] originally regarded k_4 as negligible and simplified Equation 2 accordingly,

$$K = \frac{M(T) - r_e M_e(T)}{\int_0^T C_p(t)\,dt} \quad (3)$$

where K is the product $V_f k_3$ and r_e is an "exchange ratio", equal to the ratio $k_2/(k_2 + k_3)$. The transition from Equation 1 to Equation 3 represents removal of the point of view from the precursor pool to the circulation.

Others have not regarded k_4 as negligible, particularly in primate brain, and have subsequently solved Equation 2 with more constants, but this raised the question of the relationship between the unidirectional rate of deoxyglucose phosphorylation and the net rate of glycolysis which is considered as item (3) on the list of issues given below. Generally, the question has been addressed by reference to measurements that show that dephosphorylation of glucose must be negligible ($\Phi = 1$), even if dephosphorylation of deoxyglucose is significant.

The form of Equation 3 approaches a linear relationship between the accumulated radioactivity in brain and the time–concentration integral in the circulation. The clearance K is the slope of this relationship, and the product $r_e M_e(T)$ the intercept. The linearity can be illustrated more dramatically by normalizing all terms in Equation 3 by the concentration of the tracer at the time of the limit of integration [15],

$$K = \frac{V(T) - r_e \dfrac{M_e(T)}{C_p(t)}}{\Theta(T)} \quad (4)$$

where $V(T)$ is the ratio $M(T)/C_p(T)$ which represents a volume of distribution, and $\Theta(T)$ is the normalized integral which has unit of time but equals real time only when $C_p(t)$ is constant. The ratio $M_e(T)/C_p(T)$ eventually (e.g. at infinity, or when plasma levels remain approximately constant with time) reaches a constant value equal to the magnitude of V_f.

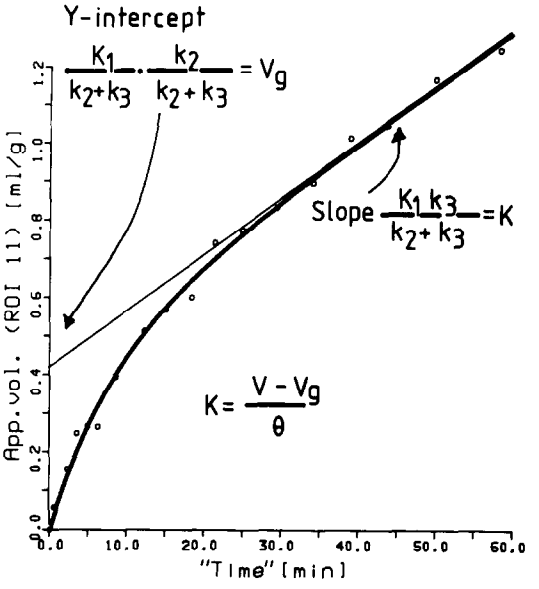

Fig. 1. Accumulation of radioactivity in cortical gray matter of human brain after intravenous injection of ^{18}fluorodeoxyglucose, plotted according to Equation 4. Abscissa: normalized time–concentration integral (min). Ordinate: apparent volume of distribution (ml/g). Ordinate intercept represents functional precursor pool volume. Note that this volume is lower than the steady-state distribution volume, as shown in Equation 4. From Gjedde *et al.* [48].

Figure 1 shows the accumulation of radioactivity derived from deoxyglucose labeled with ^{18}fluorine in the human brain, plotted according to Equation 4. When plotted in this manner, the data reveal how the value of K is obtained after subtraction of the intercept from the total volume of distribution of the label in brain.

The immeasurable quantity $M_e(T)$ is left in Equation 4. Sokoloff *et al.* considered several ways to reduce the impact of this quantity by reducing the magnitude of the intercept in those studies in which only one point on the curve is known. In these studies, the value of $M_e(T)$ must be calculated by convolution of the blood curve, and the value of r_e must be known in advance, both calculations made on the basis of the separately determined coefficients K_1, k_2, and k_3 [3].

First, since both $M_e(T)$ and r_e are functions of k_3 as well as of k_2, it can be argued that the intercept depends on one of the coefficients that the method purports to measure, i.e. k_3, but Sokoloff *et al.* wisely chose a glucose analog, the k_3 value of which is so low compared to the value of k_2 that r_e reasonably can be assumed to be unity. This particular point is an important argument in favor of using a glucose analog with a comparatively low rate of phosphorylation and is one of the reasons that glucose itself may not qualify. For glucose itself, labeled at any or all atoms, the intercept is a sensitive function of the phosphorylation rate and hence unknown unless specifically measured. In Table 1, r_e values

Table 1. Transfer coefficients and distribution volumes of glucose and glucose analogs in rat brain

Analog	K_1 ($ml \cdot g^{-1} \cdot min^{-1}$)	k_2 (min^{-1})	k_3 (min^{-1})	V_f (ml/g)	r_e
Fluorodeoxyglucose	0.31	0.60	0.13	0.42	0.82
2-Deoxyglucose	0.28	0.55	0.08	0.45	0.87
Glucose	0.19	0.36	0.26	0.30	0.56
3-*O*-Methylglucose	0.17	0.33		0.51	

From Fuglsang *et al.* [21]. Values are from a lightly anesthetized rat in which transendothelial glucose transport capacity was 4 $\mu mol \cdot g^{-1} \cdot min^{-1}$, glucose metabolic rate 0.7 $\mu mol \cdot g^{-1} \cdot min^{-1}$, arterial plasma glucose level 9 mM, and cerebral plasma flow 0.5 $ml \cdot g^{-1} \cdot min^{-1}$. The Michaelis constants (K_t) are those shown in Table 3.

are listed for glucose and the two metabolic traces. Judging from the proximity of r_e to unity, deoxyglucose is the tracer most qualified to trace glucose metabolic rate.

Equally important, Sokoloff *et al.* specifically extended the duration of accumulation so far that inaccuracies of the calculation of $M_e(T)$ could reasonably be assumed to be negligible in comparison with the magnitude of $M(T)$. Thus, in most cases it is sufficient to replace the $M_e(T)/C_p(T)$ ratio with the value of V_f for deoxyglucose listed in Table 1,

$$K = \frac{V(\infty) - r_e V_f}{\Theta(\infty)} \tag{5}$$

where ∞ denotes the prescribed duration of the circulation of the tracer, i.e. 45 min.

Four objections are often directed at the use of deoxyglucose to trace the metabolic activity of the brain: (1) deoxyglucose-6-phosphate and certain other metabolites of deoxyglucose do not remain trapped forever, (2) brain tissue does not always use glucose and deoxyglucose at the same relative rates, (3) the rates of glucose phosphorylation, glycolysis, and energy metabolism differ, also in steady-state, and (4) no steady-state prevails in the period of observation. These questions will be considered separately.

Stability of deoxyglucose metabolites

Figure 1 shows that the accumulation of deoxyglucose-derived label proceeds linearly in the human brain for at least 30 min after administration of the tracer. This observation suggests that little label is returned to deoxyglucose from the total pool of metabolites. The brain content of deoxyglucose-6-phosphate changes continuously as the result of phosphorylation of deoxyglucose, metabolism of deoxyglucose-6-phosphate to other metabolites, and dephosphorylation. Metabolism of deoxyglucose-6-phosphate to other stable metabolites does not invalidate assumptions behind the method, but dephosphorylation, if significant, would lead to underestimation of the hexokinase activity. Thus, it is important to distinguish carefully between observations of loss of deoxyglucose-6-phosphate that may or may not be important, and specific observations of dephosphorylation which are always of concern.

The rate constant for dephosphorylation would not be easy to estimate accurately if significant fractions of the labeled metabolites were not, in fact, deoxyglucose-6-phosphate. However, the exact magnitude might not be important in such cases because only a smaller fraction of the metabolites would be subject to dephosphorylation. This problem originally misled Sokoloff *et al.*, on the basis of observations made 17–24 hr after administration, to suggest a lower value of k_4 (i.e. negligible) than was later estimated [3]. The conclusion that the labeled metabolites *in toto* were stable was correct, however [16]. At times within the prescribed 30- to 45-min duration of tracer circulation, there is general agreement that deoxyglucose-6-phosphate is the predominant metabolite.

The coefficient of dephosphorylation is k_4 in Equation 2. Solving Equation 2 for all coefficients (K_1, k_2, k_3, and k_4) by positron tomography of human subjects, Phelps *et al.* [17] arrived at a value of k_4 (average 0.007 min^{-1} in gray matter) close to 10% of the average of k_3 (0.062 min^{-1}). Values of k_4 of this magnitude not only do not influence the linearity of data plotted as in Fig. 1 noticeably but are also difficult to distinguish statistically from solutions of Equation 3 in which the value of k_4 *a priori* is fixed at zero. With an improved solution in which radioactivity remaining in the circulation of the brain was taken into account, Hawkins *et al.* [18] obtained estimates of k_3 and k_4 in human gray matter that averaged 0.064 min^{-1} and 0.0075 min^{-1}, respectively, and thus confirmed the earlier estimates.

Attempts to measure the magnitude of k_4 in rats have been much more feeble because of experimental limitations. In humans, positron tomography allows continuous recording of brain radioactivity for some time. In rats, each point on curves such as that shown in Fig. 1 must come from a separate study with the consequent variation. The data are rarely so accurate that meaningful distinctions can be made between solutions employing four constants and solutions employing only three. Only in one instance, discussed below, was positron emission detection actually accomplished in rats [19].

A priori selecting a solution employing four coefficients, Huang and Veech [20] reported k_3 and k_4 values for deoxyglucose in the rat that averaged 0.114 min^{-1} and 0.051 min^{-1}, but the variation in the data was enormous, and no attempt was made to examine the discriminatory power of the solution. The result is at variance with several studies reporting excellent solutions of Equation 3 with only three constants [3, 15, 19, 21], as well as with steady-state

solutions of Equation 2. Recent estimates of the coefficients for glucose and several glucose analogs are listed in Table 1 for rat, and in Table 2 for humans.

By detection of positron emission from the brain of rats injected with ^{18}fluoride-labeled deoxyglucose, Redies *et al.* [19] also solved Equation 2 for four constants and obtained estimates of k_3 and k_4 of fluorodeoxyglucose in gray matter that averaged 0.082 min^{-1} and 0.009 min^{-1}, in excellent agreement with the human studies quoted above. As above, the magnitude of k_4 was only 10% of the value of k_3.

Although the theoretical basis for examination of the magnitude of k_4 at steady-state is weak, as discussed above, steady-state solutions of Equation 2 fortuitously provide similar results, as originally reported by Sokoloff *et al.* on the basis of studies performed 17–24 hr after administration of the tracer [3]. Equation 2 yields the following steady-state distribution volume of deoxyglucose-derived label in brain [22],

$$V(\infty) = \frac{K_1}{k_2}\left(1 + \frac{k_3}{k_4}\right) \qquad (6)$$

In Lassen and Gjedde [22], the steady-state distribution volume of deoxyglucose-derived label in brain, determined 24 hr after administration of the tracer, was close to 10 ml/g in awake rats, and in a new series studied 20 hr after injection, the steady-state distribution of deoxyglucose-derived label was close to 5 ml/g in anesthetized rats, consistent with a k_3/k_4 ratio in excess of 10. At that time, however, 37% of the label was trapped in metabolites other than deoxyglucose-6-phosphate.

Nelson *et al.* [23] and Nakada *et al.* [24] have shown that deoxyglucose-6-phosphate is slowly metabolized to glycogen, glycoproteins, phosphogluconate, and phosphogluconolactone. The formation of these metabolites may explain the loss of deoxyglucose-6-phosphate revealed by nuclear magnetic resonance [24, 25]. In both cases, however, the administered dose of deoxyglucose exceeded 2 mol/kg to permit detection of deoxyglucose-6-phosphate in the millimolar range by nuclear magnetic resonance of brain. The resulting plasma concentrations were not reported but they may have been as high as 40–50 mM, or ten times the native glucose concentration, and they therefore invalidate any conclusions drawn about the physiology of the brain.

In addition to the heterogeneity of the deoxyglucose metabolite pool, there are indications that k_4 is a variable because dephosphorylation by brain phosphatase may be a multi-compartmental process [12] involving transport of deoxyglucose-6-phosphate across reticular membranes prior to dephosphorylation. This observation would further jeopardize conclusions drawn about the magnitude of k_4 at later times.

In conclusion, there are no compelling reasons to include a term for dephosphorylation in solutions of Equation 3 that do not extend beyond 45 min.

Lumped constant

Sokoloff *et al.* [3] recognized and discussed the fact that brain hexokinase phosphorylates glucose and deoxyglucose at different rates. They also measured the ratio between the net extraction fractions of deoxyglucose and glucose and identified the six terms that influence this ratio and of which the ratio is composed (hence the name "lumped constant"). All terms, with the exception of Φ (see below), are ratios of deoxyglucose to glucose.

The component symbolized by λ is the most important because it is sensitive to departures from the physiologic state of the subject. The left-hand side of Equation 2 yields the definition of the lumped constant as the product of two ratios, the phosphorylation ratio (Π) between the values of k_3 for deoxyglucose and glucose, and the distribution ratio (λ) between the values of V_f for deoxyglucose and glucose [26], divided by a term correcting for dephosphorylation of glucose (Φ),

$$LC = \lambda\Pi/\Phi \qquad (7)$$

The phosphorylation ratio (Π) incorporates four of the six components of the lumped constant as the ratio between two separate ratios that include the ratio between the maximal phosphorylation velocities (V_{max}) of glucose and deoxyglucose (or fluorodeoxyglucose), and the ratio between the Michaelis constants (K_m) of the two substrates of brain hexokinase. Because these ratios are known and probably do not vary, Π is a constant,

$$\Pi = \frac{V^*_{max}\,K_m}{V_{max}\,K^*_m} = \frac{k^*_3}{k_3} \qquad (8)$$

where the asterisks refer to deoxyglucose.

The fifth component of the lumped constant, the distribution ratio (λ), is the ratio between the apparent volumes of distribution of deoxyglucose and glucose that depend on the relative velocities of transport across the blood–brain barrier and phosphorylation that in turn depend on the Michaelis constants for transport and phosphorylation and hence on concentrations of native glucose in blood and brain [15].

The Michaelis constants for phosphorylation and blood–brain barrier transport of glucose, deoxy-

Table 2. Gray matter transfer and phosphorylation coefficients in humans, measured by 3-parameter solution of operational equation

Tracer	K_1	k_2	k_3	V_f	r_e	[Ref.]
Glucose	0.07	0.24	0.18	0.17	0.57	[49]
Deoxyglucose	0.09	0.22	0.11	0.28	0.68	[50]
Fluorodeoxyglucose	0.11	0.15	0.07	0.48	0.67	[34]
	0.10	0.23	0.07	0.35	0.76	[26]
	0.08	0.15	0.10	0.34	0.60	[51]

Table 3. Michaelis constants, distribution ratios, and lumped constants for glucose and glucose analogs in rat brain

Analog	Blood–brain barrier K_t (mM)	Blood–brain barrier λ (ratio)	Hexokinase reaction K_m (mM)	Hexokinase reaction Π (ratio)	LC
Fluorodeoxyglucose	3	1.6	0.10	0.5	0.78
2-Deoxyglucose	4	1.6	0.17	0.3	0.48
Glucose	7		0.05		

From Fuglsang *et al.* [21]. LC was calculated for $\Phi = 1$.

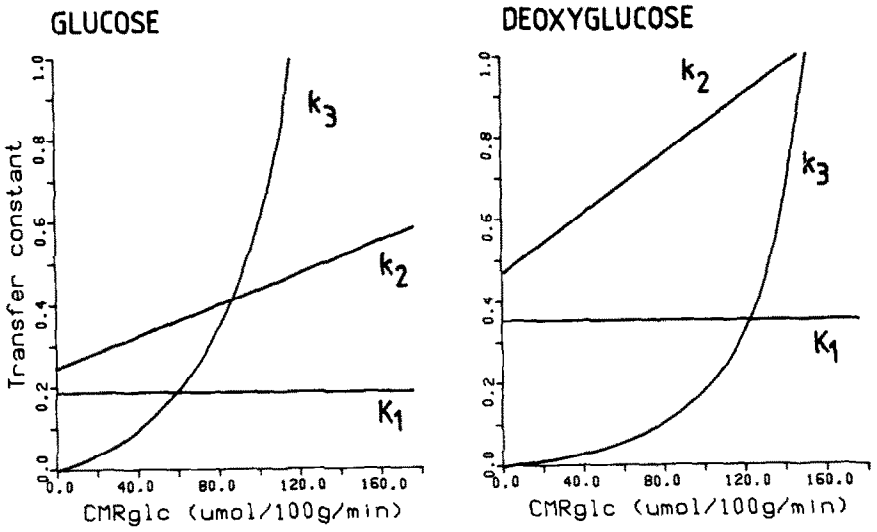

Fig. 2. Changes of coefficients k_2 and k_3 for glucose and deoxyglucose as functions of the brain glucose metabolic rate in a simulated case in which transendothelial transport and blood flow do not change in proportion to glucose metabolic rate. Abscissae: cerebral glucose metabolic rate (μmol·(100 g)$^{-1}$·min^{-1}). Ordinates: fractional clearance from precursor pools (min^{-1}). From Gjedde and Diemer [28]. Baseline values are for a lightly anesthetized rat, as listed in Table 1.

glucose, and fluorodeoxyglucose in rat are listed in Table 3 with the resulting lumped constants. The important difference between glucose and deoxyglucose is not the different affinities for hexokinase but the inverse relationship between the affinities of the two hexoses for phosphorylation and endothelial transport: Deoxyglucose has a lower affinity for phosphorylation but a higher affinity for transport. It is this property of deoxyglucose which is responsible for the concentration dependence of λ.

Both transport and phosphorylation coefficients depend on the glucose concentrations in plasma and brain, although the transport coefficients do not do so in the exact manner described by the equation of Michaelis and Menten [27]. The sensitivity of the coefficients k_2 and k_3 to alterations of brain glucose levels is illustrated in Fig. 2 for deoxyglucose and glucose [28]. This hypothetical change was simulated by an increase of brain glucose consumption with no compensatory increase of cerebral blood flow or transport across the blood–brain barrier. The consequent increase of the calculated value of the lumped constant is shown in Fig. 3.

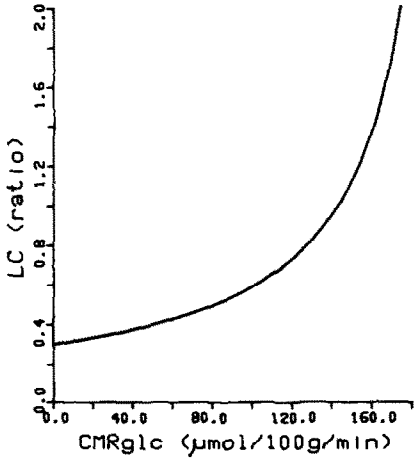

Fig. 3. Change of lumped constant for deoxyglucose as a function of cerebral metabolic rate for glucose in the simulated case described for Fig. 2. Abscissa: cerebral glucose metabolic rate (μmol·(100 g)$^{-1}$·min^{-1}). Ordinate: lumped constant (ratio). From Gjedde and Diemer [28].

Similar increases of the lumped constant can be calculated after simulated reduction of cerebral blood flow, reduction of the maximal transport capacity (T_{max}) of the brain endothelium, or reduction of the glucose concentration in blood. Any impairment of the balance between the supply and demand of glucose must favor the phosphorylation of deoxyglucose over that of glucose and hence elevate the lumped constant.

Almost by definition, the balance between supply and demand never fails under normal, physiological conditions and under these conditions the observed lumped constant has remained invariant. The balance is preserved by adjustment of blood flow and glucose transport to the prevailing demand to maintain a normal glucose content in the brain tissue. In pathologic conditions, adjustment of glucose delivery to glucose demand may fail and glucose levels in brain decline with subsequent increase of the value of the lumped constant. The most likely pathologic conditions include hypoglycemia, ischemia/oligemia of brain or regions of the brain, inhibition of glucose transport across the brain endothelium, or marked elevations of brain glucose consumption. Disruptions of the blood–brain barrier do not limit glucose delivery to the tissue (and may in fact enhance delivery) and therefore need not affect the value of the lumped constant [18].

The list of imbalances between supply and demand of glucose also represents the few cases in which changes of the lumped constant actually have been observed: hypoglycemia alone [29, 30], hypoglycemia in combination with seizures [31], seizures alone [28], and infarcts [13, 26, 32, 33]. With few exceptions, the changes have been moderate as befits a system on which the life of the subject depends. In most cases of changes of the lumped constant, the changes were calculated rather than measured, or measured in a simplified way, with the exception of the measurements by Suda *et al.* [30] of the changes of the lumped constant in hypoglycemia.

Despite the excellent theoretical basis for the suspicion that the lumped constant must change in abnormal conditions, good experimental evidence for the change is lacking. Sokoloff *et al.* [3] originally demanded that the lumped constant be measured as the ratio between the net extraction fractions of deoxyglucose and glucose in steady-state experiments in which deoxyglucose levels in arterial blood were kept constant by programmed infusion of radioactive deoxyglucose. Unfortunately, this approach is the only direct, model-independent measure of the lumped constant, but it yields no regional value for the lumped constant and is not easily performed in humans. Only recently have Reivich *et al.* [34] actually measured lumped constants for fluorodeoxyglucose and deoxyglucose in human brain by the model-independent method originally prescribed by Sokoloff *et al.* [3], as shown in Table 4.

Instead, three different calculations of the lumped constant have been proposed for regional studies in humans where the "correct" approach cannot be used.

Pardridge *et al.* [35, 36] proposed a nomogram relating blood and brain glucose to the value of the lumped constant but ignored the changes of transport capacity and blood flow that help maintain a constant glucose level in brain. Phelps *et al.* [37] proposed the use of the k_3/k_2 ratio of deoxyglucose as an index of the relationship between transport and phosphorylation of deoxyglucose which depends on the glucose level in brain and hence qualifies as an index of the lumped constant. Finally, Gjedde [15] and Gjedde and Diemer [38] proposed using the K_1/k_2 ratio determined with 3-*O*-methylglucose as an index of the brain glucose content and hence of the lumped constant. The former ratio in particular requires excellent regression statistics for accurate estimates of k_2 and the latter ratio requires an extra series of scans or autoradiograms, and both lead to an actual number for the lumped constant only on the basis of certain assumptions or *ad hoc* formulae.

In conclusion, the question of the lumped constant does not arise from an error in the model. Sokoloff *et al.* emphasized from the very beginning that pathological states required specific confirmation of fulfillment of the assumptions underlying the method, as well as measurement of the lumped constant as a necessary precaution. In normal, physiologic states, the lumped constant has been shown to deviate very little from the published values listed in Table 4, as predicted by Sokoloff *et al.* [3].

Futile cycles and the Pasteur Effect

The sixth component of the lumped constant is Φ, the fraction of phosphorylated glucose which enters the subsequent steps of glycolysis. Originally, Φ referred only to the fraction of phosphorylated glu-

Table 4. Lumped constants measured in different mammals

Species	Condition	Deoxyglucose	Fluorodeoxyglucose
Wistar rat	Neonatal	0.61 [21]	0.96 [21]
	Anesthetized	0.52 [52]	0.85 [52]
	Anesthetized	0.51 [3]	
	Anesthetized	0.48 [21]	0.78 [21]
	Conscious	0.46 [3]	
Cat	Anesthetized	0.41 [53]	
Dog	Neonatal	0.56 [54]	
Sheep	Fetal	0.42 [55]	
	Neonatal	0.38 [55]	
Pig	Anesthetized	0.52 [56]	
Rhesus monkey	Conscious	0.34 [57]	
Human	Conscious	0.56 [34]	0.52 [34]

cose subject to the action of brain isomerase, but similar fractions may apply to the later steps of glycolysis and may also apply to the fraction of glucose, once phosphorylated, which is finally oxidized to carbon dioxide. In this sense, the term Φ is flexible and can assume any empirically derived value, depending on the particular definition used. Also in this sense, Φ is the only component of the lumped constant which has not been given a specific kinetic description.

If Φ were very different from unity, a substantial fraction of glucose-6-phosphate would be returned to the glucose pool by a phosphatase reaction. Above, many arguments were used against the presence of phosphatase activity in brain sufficient to dephosphorylate 2-deoxyglucose quantitatively. Yet, Huang and Veech [39] published evidence allegedly in favor of 30% dephosphorylation of glucose-6-phosphate, such that only 70% of the glucose phosphorylated by hexokinase would continue as fructose-6-phosphate.

If this conclusion were confirmed, deoxyglucose-determined glucose phosphorylation rates would be too high if deoxyglucose recycling did not occur, or (perhaps) too low if deoxyglucose were recycled as well. According to the hypothesis, the 30% of the glucose phosphorylated by brain hexokinase goes through a "futile" cycle of phosphorylation and dephosphorylation.

The hypothesis of futile glucose cycling at the hexokinase step fails because the known concentrations of glucose and glucose-6-phosphate in brain are inconsistent with significant cycling. The concentrations imply that at least 90% of the phosphorylated glucose must continue down the glycolytic path. The fraction of glucose-6-phosphate that undergoes isomerization to fructose-6-phosphate equals the following ratio [26],

$$\Phi = 1 - \frac{k_4 C_{G6P}}{k_3 C_{glc}} \qquad (9)$$

where the concentration of glucose in brain (C_{glc}) exceeds that of glucose-6-phosphate (C_{G6P}) by at least an order of magnitude. Thus, even if the values of k_3 and k_4 were similar, Φ would not be less than 0.90 and much higher, of course, if k_4 for glucose were only 10% of k_3 as in the case of deoxyglucose.

The problem of magnitude of Φ is not identical to the problem of the value of the coefficient k_4 for deoxyglucose. Since deoxyglucose-6-phosphate accumulates rather than being further metabolized, even a low k_4 relative to k_3 could be significant. On the other hand, since glucose continues down the glycolytic path, even a high value of k_4 relative to k_3 might not have measurable consequences.

In the initial description of the deoxyglucose method, no separate value was assigned to Φ, although the authors argued that it must be close to unity if brain tissue lacks the capacity to hydrolyze glucose-6-phosphate. It is possible to estimate the value of Φ, however, by inverting Equation 7 defining the lumped constant,

$$\Phi = \lambda\Pi/LC \qquad (10)$$

If the $\lambda\Pi$ product and the LC fraction are derived independently, their ratio provides an estimate of Φ. Table 3 lists values of λ and Π obtained by regression analysis of tracer uptake and by measurements of hexokinase activities *in vitro*. When the product of these values is divided by the ratio between the extraction fractions of deoxyglucose and glucose determined by Sokoloff *et al.* in the steady-state, a result close to unity is obtained [3]. Estimates of Φ in human brain also confirm the prediction of a magnitude close to unity [32].

Nelson *et al.* [40] repeated the experiments by Huang and Veech [39] that led to the belief in a "futile" cycle of glucose phosphorylation and dephosphorylation. The careful repetition revealed an error in the procedure used by Huang and Veech to separate ^{3}H- and ^{14}C-labeled glucose. When correctly separated, the concentrations of glucose in plasma labeled with the two different isotopes did not diverge as a function of time. Thus, there is at present no evidence in favor of, and considerable evidence against, futile cycling at the brain hexokinase step. The only step with real futile cycling of glucose is the transendothelial transport at which 50% of the transported glucose is returned to the bloodstream.

A related but not identical issue is that of breakdown of glucose to end-metabolites other than carbon dioxide. If energy metabolism is defined as the rate of carbon dioxide production, any deviation of glucose moieties to products other than CO_2 (e.g. lactate) would also lead to deoxyglucose-determined rates of glucose phosphorylation that would appear to be higher than the energy metabolic rate of the brain calculated on the basis of a normal ratio between oxygen and glucose metabolic rates. Changes of glucose breakdown occur in hypoxia as the Pasteur Effect and in hyperglycosis as the Crabtree Effect. These effects reflect abnormalities, but there is increasing evidence that mismatches between glucose and oxygen metabolic rates may occur normally as well.

It is possible that there are tissues in which the rate of glucose phosphorylation significantly exceeds the rate associated with oxidative phosphorylation (e.g. retina). In these tissues, the deoxyglucose-determined rate of glucose phosphorylation represents some degree of aerobic or non-aerobic glycolysis, depending on the prevailing oxygen tension, which may be low in the inner layers of the retina. Neurons and glia may also differ in the degree of aerobic glycolysis during activation, and neurons themselves may differ ("white" and "red" neurons) as muscle cells differ.

Recently, simultaneous measurements of blood flow and oxygen consumption in the human brain by positron tomography have revealed a dissociation of the two variables during somatosensory stimulation, suggesting that energy requirements during activation are met by partial aerobic glycolysis [41].

In conclusion, there is no doubt that the measured rate of glucose phosphorylation is not a measure of the rate of oxidative phosphorylation but neither is the rate of oxidative phosphorylation necessarily a measure of the energy metabolic rate. Only the erroneous view that the glucose phosphorylation rate represents the "energy metabolism" of the brain

could possibly lead to confusion about this point. The unlikely suggestion that major fractions of brain glucose undergo "futile" cycling has not stood up to careful scrutiny.

Steady-state

The steady-state requirements of the method are not fulfilled if the glucose metabolic rate of the brain changes during the study. The question is one of the time frame of the changes. Nobody could safely argue that activation of the brain is not associated with second-to-second or minute-to-minute fluctuations of many variables that characterize the energy metabolic state of the brain, e.g. tissue oxygen tension, microflow, and ion concentrations. What concerns the user of the deoxyglucose method is not these rapid changes (although they are of extreme interest) because they remain outside the scope of the method. Rather, the user is concerned about significant baseline changes of the physiologic state of the animal or human subject that may occur and persist during a major part of the duration of the study. When that happens, the deoxyglucose-determined phosphorylation rate is some ill-defined time average of the rates prevailing during the 45-min period of circulation of the tracer.

For example, Crosby *et al.* [42] reported the effects of ketamine on regional brain glucose phosphorylation, using the deoxyglucose method. During the 45-min circulation period, the behavior of the animals changed from cataleptic-like unresponsiveness to side-to-side head rocking and frantic, agitated hyperactivity. Crosby and Sokoloff [43] argued that accumulation of 2-deoxyglucose-6-phosphate was heavily weighted towards the first 15 min of the circulation and thus reflected events during the early period of inactivity. After the first 15 min, accumulation of tracer slowed down because the plasma level declined to low levels and thus did not invalidate the conclusions drawn from the study.

The problems of steady-state have prompted the search for methods of shorter required circulation of the tracer. The glucose method of Gaitonde [9], adopted, among others, by Hawkins *et al.* [44, 45] and Blomqvist *et al.* [46], in principle does not permit durations longer than some short time, e.g. 10 min, depending on the position of the label, without correction for egress of CO_2, because labeled CO_2 is lost from the tissue. The safe duration remains in doubt and may vary with the condition under investigation, i.e. the higher the suspected rate of phosphorylation, the shorter the safe duration of circulation. In addition, the correction for unphosphorylated glucose (the second part of the numerator in Equation 3) is relatively no smaller a term for glucose than for deoxyglucose.

At 10 min, available evidence indicates that 10% of radioactivity in brain derived from glucose labeled in the C6-position remains in the form of glucose. This percentage is as large as the percentage of radioactivity derived from labeled deoxyglucose that remains in the form of unphosphorylated deoxyglucose 45 min after administration of labeled deoxyglucose.

Unlike deoxyglucose, the exact amount of radioactive glucose to be subtracted is sensitive to the magnitude of the unknown glucose phosphorylation rate. However, glucose, particularly when labeled in the C6-position, may offer the advantage that no lumped constant need be known if or when a correct compensation for lost CO_2 can be made [46].

An alternative to the use of glucose to solve the problem of non-steady-state is to shorten the circulation of labeled deoxyglucose and introduce a marker for unmetabolized deoxyglucose. The rate of phosphorylation of deoxyglucose is particularly slow compared to its efflux from brain. For this reason, methylglucose which is not metabolized can trace the distribution of unmetabolized deoxyglucose as proposed by Gjedde [15] and performed by Siemkowicz *et al.* [47] and Nedergaard *et al.* [33]. This approach allows the circulation of deoxyglucose to be shortened to 10 min, except in situations in which circulation to the tissue is retarded severely.

In conclusion, maintaining a steady-state is a real problem for brain metabolic studies but not because of shortcomings of the deoxyglucose model. Major changes of the brain physiologic state of the subject probably invalidate the measurements if the changes occur in the first 15 min after administration of labeled deoxyglucose, as emphasized by Crosby and Sokoloff [43]. Alternative uses of the deoxyglucose method in non-steady-states have been proposed, and major infractions of the steady-state rule have not appeared in the literature.

Evaluation

The basic precepts of the deoxyglucose method overwhelmingly appear to be upheld, perhaps with the exception of the requirement of maintaining a steady-state in certain activation states. The lumped constant is a coefficient which has shown remarkable constancy under normal physiological conditions, probably because the concentration of glucose in brain is a controlled variable, and several methods are available for the safe estimation of lumped constant variations when glucose concentrations in brain may change.

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